

WHAT IS CLAIMED IS:

1           1.       A method of predicting the likelihood of long-term survival of a breast  
2 cancer patient without the recurrence of breast cancer, comprising determining the  
3 expression level of one or more prognostic RNA transcripts or their expression products  
4 in a breast cancer tissue sample obtained from said patient, normalized against the  
5 expression level of all RNA transcripts or their products in said breast cancer tissue  
6 sample, or of a reference set of RNA transcripts or their expression products, wherein the  
7 prognostic RNA transcript is the transcript of one or more genes selected from the group  
8 consisting of: TP53BP2, GRB7, PR, CD68, Bcl2, KRT14, IRS1, CTSL, EstR1, Chk1,  
9 IGFBP2, BAG1, CEGP1, STK15, GSTM1, FHIT, RIZ1, AIB1, SURV, BBC3, IGF1R,  
10 p27, GATA3, ZNF217, EGFR, CD9, MYBL2, HIF1 $\alpha$ , pS2, ErbB3, TOP2B, MDM2,  
11 RAD51C, KRT19, TS, Her2, KLK10,  $\beta$ -Catenin,  $\gamma$ -Catenin, MCM2, PI3KC2A, IGF1,  
12 TBP, CCNB1, FBXO5, and DR5,

13               wherein expression of one or more of GRB7, CD68, CTSL, Chk1, AIB1,  
14 CCNB1, MCM2, FBXO5, Her2, STK15, SURV, EGFR, MYBL2, HIF1 $\alpha$ , and TS  
15 indicates a decreased likelihood of long-term survival without breast cancer recurrence,  
16 and the expression of one or more of TP53BP2, PR, Bcl2, KRT14, EstR1, IGFBP2,  
17 BAG1, CEGP1, KLK10,  $\beta$ -Catenin,  $\gamma$ -Catenin, DR5, PI3KCA2, RAD51C, GSTM1,  
18 FHIT, RIZ1, BBC3, TBP, p27, IRS1, IGF1R, GATA3, ZNF217, CD9, pS2, ErbB3,  
19 TOP2B, MDM2, IGF1, and KRT19 indicates an increased likelihood of long-term  
20 survival without breast cancer recurrence.

1           2.       The method of claim 1 comprising determining the expression level of at  
2 least two of said prognostic RNA transcripts or their expression products.

1           3.       The method of claim 1 comprising determining the expression level of at  
2 least 5 of said prognostic RNA transcripts or their expression products.

1           4.       The method of claim 1 comprising determining the expression level of at  
2 least 10 of said prognostic RNA transcripts or their expression products.

1           5.       The method of claim 1 comprising determining the expression level of at  
2 least 15 of said prognostic transcripts of their expression products.

1           6.       The method of claim 1 wherein the breast cancer is invasive breast  
2 carcinoma.

1           7.       The method of claim 1 wherein the expression level of one or more  
2 prognostic RNA transcripts is determined.

1           8.       The method of claim 1 wherein said RNA is isolated from a fixed, wax-  
2 embedded breast cancer tissue specimen of said patient.

1           9.       The method of claim 1 wherein said RNA is isolated from core biopsy  
2 tissue or fine needle aspirate cells.

1           10.      An array comprising polynucleotides hybridizing to two or more of the  
2 following genes:  $\alpha$ -Catenin, AIB1, AKT1, AKT2,  $\beta$ -actin, BAG1, BBC3, Bcl2, CCNB1,  
3 CCND1, CD68, CD9, CDH1, CEGP1, Chk1, CIAP1, cMet.2, Contig 27882, CTSL, DR5,  
4 EGFR, EIF4E, EPHX1, ErbB3, EstR1, FBXO5, FHIT1 FRP1, GAPDH, GATA3, G-  
5 Catenin, GRB7, GRO1, GSTM1, GUS, HER2, HIF1A, HNF3A, IGF1R, IGFBP2,  
6 KLK10, KRT14, KRT17, KRT18, KRT19, KRT5, Maspin, MCM2, MCM3, MDM2,  
7 MMP9, MTA1, MYBL2, P14ARF, p27, P53, PI3KC2A, PR, PRAME, pS2,  
8 RAD51C, 3RB1, RIZ1, STK15, STMY3, SURV, TGFA, TOP2B, TP53BP2, TRAIL, TS,  
9 upa, VDR, VEGF, and ZNF217.

1           11.      The array of claim 10 comprising polynucleotides hybridizing to at least 3  
2 of said genes.

1           12.      The array of claim 10 comprising polynucleotides hybridizing to at least 5  
2 of said genes.

1           13.      The array of claim 10 comprising polynucleotides hybridizing to at least  
2 10 of said genes.

1           14.    The array of claim 10 comprising polynucleotides hybridizing to the  
2 following genes: TP53BP2, GRB7, PR, CD68, Bcl2, KRT14, IRS1, CTSL, EstR1, Chk1,  
3 IGFBP2, BAG1, CEGP1, STK15, GSTM1, FHIT, RIZ1, AIB1, SURV, BBC3, IGF1R,  
4 p27, GATA3, ZNF217, EGFR, CD9, MYBL2, HIF1 $\alpha$ , pS2, RIZ1, ErbB3, TOP2B,  
5 MDM2, RAD51C, KRT19, TS, Her2, KLK10,  $\beta$ -Catenin,  $\gamma$ -Catenin, MCM2, PI3KC2A,  
6 IGF1, TBP, CCNB1, FBXO5 and DR5.

1           15.    The array of claim 10 or claim 14 wherein said polynucleotides are  
2 cDNAs.

1           16.    The array of claim 15 wherein said cDNAs are about 500 to 5000 bases  
2 long.

1           17.    The array of claim 10 or claim 14 wherein said polynucleotides are  
2 oligonucleotides.

1           18.    The array of claim 17 wherein said oligonucleotides are about 20 to 80  
2 bases long.

1           19.    The array of claim 10 or claim 14 wherein the solid surface is glass.

1           20.    The array of claim 19 which comprises about 330,000 oligonucleotides.

1           21.    A method of predicting the likelihood of long-term survival of a patient  
2 diagnosed with invasive breast cancer, without the recurrence of breast cancer,  
3 comprising the steps of:

4               (1)    determining the expression levels of the RNA transcripts or the  
5 expression products of genes or a gene set selected from the group consisting of

6       (a)    TP53BP2, Bcl2, BAD, EPHX1, PDGFR $\beta$ , DIABLO, XIAP, YB1, CA9, and  
7           KRT8;

8       (b)    GRB7, CD68, TOP2A, Bcl2, DIABLO, CD3, ID1, PPM1D, MCM6, and WISP1;

9       (c)    PR, TP53BP2, PRAME, DIABLO, CTSL, IGFBP2, TIMP1, CA9, MMP9, and  
10           COX2;

11       (d)    CD68, GRB7, TOP2A, Bcl2, DIABLO, CD3, ID1, PPM1D, MCM6, and WISP1;

- 12 (e) Bcl2, TP53BP2, BAD, EPHX1, PDGFR $\beta$ , DIABLO, XIAP, YB1, CA9, and  
13 KRT8;
- 14 (f) KRT14, KRT5, PRAME, TP53BP2, GUS1, AIB1, MCM3, CCNE1, MCM6, and  
15 ID1;
- 16 (g) PRAME, TP53BP2, EstR1, DIABLO, CTSL, PPM1D, GRB7, DAPK1, BBC3,  
17 and VEGFB;
- 18 (h) CTSL2, GRB7, TOP2A, CCNB1, Bcl2, DIABLO, PRAME, EMS1, CA9, and  
19 EpCAM;
- 20 (i) EstR1, TP53BP2, PRAME, DIABLO, CTSL, PPM1D, GRB7, DAPK1, BBC3,  
21 and VEGFB;
- 22 (k) Chk1, PRAME, TP53BP2, GRB7, CA9, CTSL, CCNB1, TOP2A, tumor size, and  
23 IGFBP2;
- 24 (l) IGFBP2, GRB7, PRAME, DIABLO, CTSL,  $\beta$ -Catenin, PPM1D, Chk1, WISP1,  
25 and LOT1;
- 26 (m) HER2, TP53BP2, Bcl2, DIABLO, TIMP1, EPHX1, TOP2A, TRAIL, CA9, and  
27 AREG;
- 28 (n) BAG1, TP53BP2, PRAME, IL6, CCNB1, PAI1, AREG, tumor size, CA9, and  
29 Ki67;
- 30 (o) CEGP1, TP53BP2, PRAME, DIABLO, Bcl2, COX2, CCNE1, STK15, and  
31 AKT2, and FGF18;
- 32 (p) STK15, TP53BP2, PRAME, IL6, CCNE1, AKT2, DIABLO, cMet, CCNE2, and  
33 COX2;
- 34 (q) KLK10, EstR1, TP53BP2, PRAME, DIABLO, CTSL, PPM1D, GRB7, DAPK1,  
35 and BBC3;
- 36 (r) AIB1, TP53BP2, Bcl2, DIABLO, TIMP1, CD3, p53, CA9, GRB7, and EPHX1
- 37 (s) BBC3, GRB7, CD68, PRAME, TOP2A, CCNB1, EPHX1, CTSL  
38 GSTM1, and APC;
- 39 (t) CD9, GRB7, CD68, TOP2A, Bcl2, CCNB1, CD3, DIABLO, ID1, and PPM1D;
- 40 (w) EGFR, KRT14, GRB7, TOP2A, CCNB1, CTSL, Bcl2, TP, KLK10, and CA9;
- 41 (x) HIF1 $\alpha$ , PR, DIABLO, PRAME, Chk1, AKT2, GRB7, CCNE1, TOP2A, and  
42 CCNB1;
- 43 (y) MDM2, TP53BP2, DIABLO, Bcl2, AIB1, TIMP1, CD3, p53, CA9, and HER2;
- 44 (z) MYBL2, TP53BP2, PRAME, IL6, Bcl2, DIABLO, CCNE1, EPHX1, TIMP1, and  
45 CA9;

46 (aa) p27, TP53BP2, PRAME, DIABLO, Bcl2, COX2, CCNE1, STK15, AKT2, and  
 47 ID1;  
 48 (ab) RAD51, GRB7, CD68, TOP2A, CIAP2, CCNB1, BAG1, IL6, FGFR1, and  
 49 TP53BP2;  
 50 (ac) SURV, GRB7, TOP2A, PRAME, CTSL, GSTM1, CCNB1, VDR, CA9; and  
 51 CCNE2;  
 52 (ad) TOP2B, TP53BP2, DIABLO, Bcl2, TIMP1, AIB1, CA9, p53, KRT8, and BAD;  
 53 (ae) ZNF217, GRB7, TP53BP2, PRAME, DIABLO, Bcl2, COX2, CCNE1, APC4,  
 54 and  $\beta$ -Catenin,  
 55 in a breast cancer tissue sample obtained from said patient, normalized against the  
 56 expression levels of all RNA transcripts or their expression products in said breast cancer  
 57 tissue sample, or of a reference set of RNA transcripts or their products;  
 58 (2) subjecting the data obtained in step (1) to statistical analysis; and  
 59 (3) determining whether the likelihood of said long-term survival has  
 60 increased or decreased.

1 22. A method of predicting the likelihood of long-term survival of a patient  
 2 diagnosed with estrogen receptor (ER)-positive invasive breast cancer, without the  
 3 recurrence of breast cancer, comprising the steps of:  
 4 (1) determining the expression levels of the RNA transcripts or the  
 5 expression products of genes of a gene set selected from the group consisting of CD68;  
 6 CTSL; FBXO5; SURV; CCNB1; MCM2; Chk1; MYBL2; HIF1A; cMET; EGFR; TS;  
 7 STK15, IGFR1; Bcl2; HNF3A; TP53BP2; GATA3; BBC3; RAD51C; BAG1; IGFBP2;  
 8 PR; CD9; RB1; EPHX1; CEGP1; TRAIL; DR5; p27; p53; MTA; RIZ1; ErbB3; TOP2B;  
 9 EIF4E, wherein expression of the following genes in ER-positive cancer is indicative of a  
 10 reduced likelihood of survival without cancer recurrence following surgery: CD68;  
 11 CTSL; FBXO5; SURV; CCNB1; MCM2; Chk1; MYBL2; HIF1A; cMET; EGFR; TS;  
 12 STK15, and wherein expression of the following genes is indicative of a better prognosis  
 13 for survival without cancer recurrence following surgery: IGFR1; Bcl2; HNF3A;  
 14 TP53BP2; GATA3; BBC3; RAD51C; BAG1; IGFBP2; PR; CD9; RB1; EPHX1; CEGP1;  
 15 TRAIL; DR5; p27; p53; MTA; RIZ1; ErbB3; TOP2B; EIF4E.  
 16 (2) subjecting the data obtained in step (1) to statistical analysis; and  
 17 (3) determining whether the likelihood of said long-term survival has  
 18 increased or decreased.

1           23.     The method of claim 21 or 22 wherein said statistical analysis is performed  
2 by using the Cox Proportional Hazards model.

1           24.     A method of predicting the likelihood of long-term survival of a patient  
2 diagnosed with estrogen receptor (ER)-negative invasive breast cancer, without the  
3 recurrence of breast cancer, comprising determining the expression levels of the RNA  
4 transcripts or the expression products of genes of the gene set CCND1; UPA; HNF3A;  
5 CDH1; Her2; GRB7; AKT1; STMY3;  $\alpha$ -Catenin; VDR; GRO1; KT14; KLK10; Maspin,  
6 TGF $\alpha$ , and FRP1, wherein expression of the following genes is indicative of a reduced  
7 likelihood of survival without cancer recurrence: CCND1; UPA; HNF3A; CDH1; Her2;  
8 GRB7; AKT1; STMY3;  $\alpha$ -Catenin; VDR; GRO1, and wherein expression of the  
9 following genes is indicative of a better prognosis for survival without cancer recurrence:  
10 KT14; KLK10; Maspin, TGF $\alpha$ , and FRP1.

1           25.     A method of preparing a personalized genomics profile for a patient,  
2 comprising the steps of:  
3                 (a)     subjecting RNA extracted from a breast tissue obtained from the  
4 patient to gene expression analysis;  
5                 (b)     determining the expression level of one or more genes selected  
6 from the breast cancer gene set listed in any one of Tables 1-5, wherein the expression  
7 level is normalized against a control gene or genes and optionally is compared to the  
8 amount found in a breast cancer reference tissue set; and  
9                 (c)     creating a report summarizing the data obtained by said gene  
10 expression analysis.

1           26.     The method of claim 25, wherein said breast tissue comprises breast  
2 cancer cells.

1           27.     The method of claim 26 wherein said breast tissue is obtained from a  
2 fixed, paraffin-embedded biopsy sample.

1           28.     The method of claim 27 wherein said RNA is fragmented.

1           29.     The method of claim 25 wherein said report includes prediction of the  
2     likelihood of long term survival of the patient.

1           30.     The method of claim 25 wherein said report includes recommendation for  
2     a treatment modality of said patient.

1           31.     A method for amplification of a gene listed in Tables 5A and B by  
2     polymerase chain reaction (PCR), comprising performing said PCR by using an amplicon  
3     listed in Tables 5A and B and a primer-probe set listed in Tables 6A-F.

1           32.     A PCR amplicon listed in Tables 5A and B.

1           33.     A PCR primer-probe set listed in Tables 6A-F.

1           34.     A prognostic method comprising:

2                 (a)     subjecting a sample comprising breast cancer cells obtained from a  
3     patient to quantitative analysis of the expression level of the RNA transcript of at least  
4     one gene selected from the group consisting of GRB7, CD68, CTSL, Chk1, AIB1,  
5     CCNB1, MCM2, FBXO5, Her2, STK15, SURV, EGFR, MYBL2, HIF1 $\alpha$ , and TS, or  
6     their product, and

7                 (b)     identifying the patient as likely to have a decreased likelihood of  
8     long-term survival without breast cancer recurrence if the normalized expression levels of  
9     said gene or genes, or their products, are elevated above a defined expression threshold.

1           35.     A prognostic method comprising:

2                 (a)     subjecting a sample comprising breast cancer cells obtained from a  
3     patient to quantitative analysis of the expression level of the RNA transcript of at least  
4     one gene selected from the group consisting of TP53BP2, PR, Bcl2, KRT14, EstR1,  
5     IGFBP2, BAG1, CEGP1, KLK10,  $\beta$ -Catenin,  $\gamma$ -Catenin, DR5, PI3KCA2, RAD51C,  
6     GSTM1, FHIT, RIZ1, BBC3, TBP, p27, IRS1, IGF1R, GATA3, ZNF217, CD9, pS2,  
7     ErbB3, TOP2B, MDM2, IGF1, and KRT19, and

8                 (b)     identifying the patient as likely to have an increased likelihood of  
9     long-term survival without breast cancer recurrence if the normalized expression levels of  
10    said gene or genes, or their products, are elevated above a defined expression threshold.

1           36.     The method of claim 1 wherein the levels of the RNA transcripts of said  
2 genes are normalized relative to the mean level of the RNA transcript or the product of  
3 two or more housekeeping genes.

1           37.     The method of claim 34 or 35 wherein the housekeeping genes are selected  
2 from the group consisting of glyceraldehyde-3-phosphate dehydrogenase (GAPDH),  
3 Cyp1, albumin, actins, tubulins, cyclophilin hypoxanthine phosphoribosyltransferase  
4 (HRPT), L32, 28S, and 18S.

1           38.     The method of claim 34 or 35 wherein the sample is subjected to global  
2 gene expression analysis of all genes present above the limit of detection.

1           39.     The method of claim 37 wherein the levels of the RNA transcripts of said  
2 genes are normalized relative to the mean signal of the RNA transcripts or the products of  
3 all assayed genes or a subset thereof.

1           40.     The method of claim 38 wherein the level of RNA transcripts is  
2 determined by quantitative RT-PCR (qRT-PCR), and the signal is a Ct value.

1           41.     The method of claim 39 wherein the assayed genes include at least 50  
2 cancer related genes.

1           42.     The method of claim 39 wherein the assayed genes includes at least 100  
2 cancer related genes.

1           43.     The method of claim 34 or 35 wherein said patient is human.

1           44.     The method of claim 42 wherein said sample is a fixed, paraffin-embedded  
2 tissue (FPET) sample, or fresh or frozen tissue sample.

1           45.     The method of claim 42 wherein said sample is a tissue sample from fine  
2 needle, core, or other types of biopsy.



1           46.     The method of claim 42 wherein said quantitative analysis is performed by  
2     qRT-PCR.

1           47.     The method of claim 42 wherein said quantitative analysis is performed by  
2     quantifying the products of said genes.

1           48.     The method of claim 45 wherein said products are quantified by  
2     immunohistochemistry or by proteomics technology.

1           49.     The method of claim 34 further comprising the step of preparing a report  
2     indicating that the patient has a decreased likelihood of long-term survival without breast  
3     cancer recurrence.

1           50.     The method of claim 35 further comprising the step of preparing a report  
2     indicating that the patient has an increased likelihood of long-term survival without breast  
3     cancer recurrence.

1           51.     A kit comprising one or more of (1) extraction buffer/reagents and  
2     protocol; (2) reverse transcription buffer/reagents and protocol; and (3) qPCR  
3     buffer/reagents and protocol suitable for performing the method of any one of claims 1,  
4     34 and 35.